IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Michael A. Tainsky, et al. Confirmation No.: 5172

Serial No.: 10/004,587 Group Art Unit: 1631

Filed: 12/04/2001 Examiner: CLOW, Lori A.

For: NEOEPITOPE DETECTION OF DISEASE USING PROTEIN ARRAYS

Attorney Docket No.: 0788.00063

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION

I, Dr. Michael A. Tainsky, being duly sworn, do hereby state that:

1. I am a co-inventor of the above-captioned application.

2. I am skilled in the art and have worked extensively in the field of biopanning and cancer detection.

3. The present invention includes at least two novel solutions to problems that the we have found to be important impediments to assembling high density panels of tumor markers that are both accurate in diagnosing cancer yet compact enough to be technically and economically feasible.

The first problem is the occurrence of control sera with high but nonspecific activity to certain epitopes. This phenomenon causes the erroneous rejection of epitopes which are potentially useful tumor markers, because epitopes reactive with control serum are excluded from marker panels constructed through phage display. (paragraph 0088, lines 4-17).

The solution to this problem is the step of recognizing and excluding the sera of normal individuals that react strongly but nonspecifically to epitope bearing clones. This step has now been incorporated into amended claim 20. The amendment is supported by a specific embodiment of the solution, which is fully described in the specification and is itself the subject of new dependent claim 21:

the step of excluding sera which show a unimodal pattern of dual fluorescence reaction with epitope bearing clones, where one color indicates reaction with human immunoglobulin (Ig) and the other color indicates reaction to phage capsid proteins.

Essentially, this step requires that, if a phage clone stains positively for binding of human Ig of a control serum, but the staining is not sufficiently intense to form a fluorescence peak separate from that produced by immunofluorescence staining of phage capsid protein (i.e. unimodal dual fluorescence distribution, as in Figs. 8A and 9A), then that phage clone is not discarded as bearing an epitope reactive with an Ig of a normal individual. Instead, the serum is excluded from the analysis, for the unimodal fluorescence marks it as containing Ig's which bind nonspecifically to the phage clone. Phage clones are discarded only when the binding of human Ig of a control serum produces fluorescence which surpasses the immunofluorescence staining of phage capsid proteins sufficiently to produce a separate peak on a logarithmic scale (i.e. bimodal dual fluorescence distribution, as in Figs 8B and 9B).

The present amendment is supported in the disclosure in paragraph 0088. There the problem is recognized and the solution is explained, including references to Figs 8 and 9, which show the distinction between unimodal and bimodal fluorescence profiles. The construction of the histograms for determination of unimodal vs. bimodal fluorescence distribution is further explained in paragraphs 0165-0166. The unimodal vs. bimodal fluorescence analysis is used to reduce the artifactual elimination of potentially useful tumor markers in Example 1, in paragraph 0159. That is part of the analysis of epitope expression in a phage display library constructed from ovarian tumor cells.

It must be stressed that the step of recognizing and excluding the sera of normal individuals which react strongly but nonspecifically to epitope bearing clones is not a normalization step, but a step performed after normalization is complete. Normalization is provided as the part of the first major step of data analysis, "Pre processing and normalization" (Paragraphs 0077 and 0078). The purpose of normalization, explained in paragraph 0081, is to cleanse the data of artifacts such as slide contamination, differential dye incorporation, scanning and image

processing problems, and imperfect spots due to imperfect arraying, washing, drying, etc. The techniques of normalization are described in paragraph 0082. In contrast, the step of recognizing and excluding the sera of normal individuals which react strongly but nonspecifically to epitope bearing clones is performed as part of the second major step of data anlysis, "Identifying the most informative markers" (Paragraph 0079).

Neither Sioud, et al. nor Miller, et al., nor Robinson, et al. disclose disclose all of the limitations of the presently amended independent claim 20 and new dependent claim 21. Sioud, et al. teaches densitometric comparisons of phage clones with cancer patient Ig and control Ig (page 717, last paragraph, to page 718, first paragraph). . There is no recognition of the possibility of artifactual exclusion of useful epitopes because of intense but nonspecific binding of control Ig to phage, and no solution to the problem is disclosed. Miller, et al. teaches only normalization of the same type disclosed as the normalization step of the present invention. Reporter molecules are used to correct for variations in the amount of protein spotted on a solid support (page 48 lines 21-29), or to correct for variations in the amount of antibody spotted on a solid support or for variations in the association and dissociation constants of different antibodies to the same protein (page 52 line 21 to page 53 line 8). There is no recognition of the possibility of erroneous exclusion of useful epitopes because of intense but nonspecific binding of control Ig to a possible marker, and no solution to the problem is disclosed or suggested. Robinson, et al. discloses methods of two color staining of arrays in paragraphs 0070 to 0077, with paragraph 0074 focusing especially on the use of negative and positive controls. Again, there is no recognition of the possibility of erroneous exclusion of useful epitopes because of intense but nonspecific binding of control Ig to a possible marker, and no solution to the problem is disclosed or suggested.

In summary, the cited references, alone or in combination with knowledge in the art, disclose neither the general concept of recognizing and excluding the sera of normal individuals which react strongly but nonspecifically to epitope bearing clones, nor the specific embodiment of excluding sera which show a unimodal pattern of dual fluorescence reaction with epitope bearing clones. No combination of

references protects against the unnecessary elimination of useful epitopes as does the present invention. Consequently, amended claim 20 and new claim 21 are clearly patentable over the combinations of Sioud, et al. with either Miller, et al., or Robinson, et al. Reconsideration of the rejection is respectfully requested.

The second problem recognized by the applicants and solved by the invention is the problem of patient sera which react to only a small number of very specific epitopes. If the selection of the epitopes is done on statistical grounds alone, these very specific epitopes can be missed. Simply including all such epitopes, however, can result in panels of unacceptable size. This problem is an obstacle to the assembly of a high density panel of tumor markers that is both accurate in its ability to diagnose cancer yet compact enough to be technically and economically feasible.

The solution to this problem is to maximize the information content of the selected panel of markers while minimizing the number of epitopes included. This solution is now incorporated into amended claim 20. The amendment is supported by a description of the problem and solution paragraph 0089. Note that the solution is referred to therein as an "essential modification" overall procedure of identifying the most informative markers.

A specific embodiment of this solution is the subject of new dependent claim 22. It is a method for ensuring that epitopes which react solely with self serum – that is only with the serum of the patient used to isolate the epitope – are incorporated in the panel only if that self serum does not also cross react with epitopes isolated with sera of other patients. If the self serum does cross react with epitopes isolated with other sera, the epitope can be excluded. This condition permits the exclusion of some eptiopes while ensuring that the resulting array retains information from all patient sera in the sample (paragraph 0096, last line). The method that puts this rule into effect is described in paragraphs 0093-0096, which detail the construction and stepwise editing of a table of reactive clones and patient sera. The method is also described in Example 1, in paragraph 0164.

None of the cited references recognizes the problem of ensuring the inclusion of rare but informative epitopes while minimizing the number of epitopes included in the panel of markers, and none of them provide or suggest any solution. Sioud, et al. does not treat the problem as it does not disclose a high density array. Neither of the two references which provide high density arrays, i.e Miller, et al., and Robinson, et al. contemplate any practical limits on the size of marker panels resulting from their arrays, nor of the possible need to limit the inclusion of rare but possibly informative markers. Consequently, amended claim 20 and new claim 22 are clearly patentable over the combinations of Sioud, et al. with either Miller, et al., or Robinson, et al. Reconsideration of the rejection is respectfully requested.

There is an additional reason why the present invention is not rendered obvious by Sioud, et al. in view of Miller, et al. Miller, et al. teaches the creation of an array of defined monoclonal antibodies with which to detect differences in protein expression among samples of tissue and body fluid. The present invention creates an array of defined proteins or peptides with which to detect differences in anti-tumor antibody expression in body fluids of normal individuals and cancer patients. This inventive concept exactly opposes the teaching of Miller, et al. The inventive concept of Sioud, et al. also exactly opposes the teachings of Miller, et al. It entails the definition of individual proteins with which to detect differences in anti-tumor antibody expression in body fluids of normal individuals and cancer patients. The antibody-based detection method taught by Miller, et al therefore cannot be combined with the protein based detection method taught by Sioud, et al. in any useful way. Since neither the cited references alone or in combination with knowledge in the art suggest the currently claimed invention, the claims are clearly patentable over the combination.

There is an additional reason why the present invention is not rendered obvious by Sioud, et al. in view of Robinson, et al. The array disclosed by Robinson et al. is essentially an ELISA plate assay. The combination of this array with the phage display methods taught by Sioud, et al. would produce methods which would not be feasible, in terms of time and cost, for the screening of candidate marker populations of the size which can be screened by the present invention. Since

neither the cited references alone or in combination with knowledge in the art suggest the currently claimed invention, the claims are clearly patentable over the combination.

The undersigned declares further all statements made herein of his knowledge are true and that all statements made upon information and belief are believed to be true, and further that the statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12/11/09

Dr. Michael A. Tainsky